SHORT COMMUNICATION

Pentafluorobenzyl Chloroformate Derivatization for Enhancement of Detection of Amino Acids or Alcohols by Electron Capture Negative Ion Chemical Ionization Mass Spectrometry

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Pentafluorobenzyl chloroformate (PFB-chloroformate) has been utilized as a derivatization reagent to impart electron affinity and provide structurally relevant fragmentation in electron capture negative ion chemical ionization mass spectrometry (ECNICI-MS). Phenylalanine (Phe) and decanol were used as model analytes. The conditions used for their derivatization and the chromatographic and mass spectrometric properties of the derivatives are reported. Phenylalanine in aqueous solution was derivatized in one step by using PFB-chloroformate and a mixture of water, ethanol, and pyridine. The phenylalanine *N*-pentafluorobenzyloxycarbonyl ethyl ester (N-PFBC-Phe-OEt) exhibited good gas chromatographic properties and in ECNICI-MS, a dominant $[M - 181]^-$ fragment carries most of the ion current. Selected ion monitoring experiments on N-PFBC-Phe-OEt resulted in the facile detection of 400 fmol of material. Decanol was derivatized by using anhydrous conditions, and the resultant pentafluorobenzyl carbonate also exhibited a predominant $[M - 181]^-$ ion in ECNICI-MS. Initial results indicate that the ECNICI-MS molar response of the decyl pentafluorobenzyl carbonate derivative is six-fold that of the decyl pentafluorobenzoate. *(J Am Soc Mass Spectrom 1995, 6, 525–528)*

There have been several reports in the recent literature that concern the use of chloroformates for aqueous, rapid, room temperature one-step derivatization of amino acids (AA) with analysis by gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). This interest was initiated by the work of Husek [1] and more recently extended by Wang et al. [2]. The general experimental procedure is to add an aliquot of an AA in dilute acid to a mixture of water, an alcohol (ethanol), and an organic base (pyridine), followed by the addition of a chloroformate (ethyl chloroformate). The solution is then vortexed and immediately extracted with chloroform. Under these conditions, carboxylic acids are converted to ethyl esters and amines are converted to ethyl carbamates. Guanidino and aliphatic hydroxyl groups do not react with the chloroformate. The extracted derivatives are then analyzed by GC or GC-MS. This procedure is applicable to all of the AA, except arginine, which does not elute from the GC column [2].

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Electron capture negative ion chemical ionization (ECNICI) GC-MS of chloroformate AA derivatives was investigated by Vatankhah and Moini [3], who used trifluoroethanol in the reaction mixture to increase electron affinity of the resultant trifluoroethyl ester derivatives. Although the detection limit of the trifluoroethyl esters was improved relative to the ethyl esters, a dramatic increase in sensitivity in negative ion chemical ionization (NICI) versus positive ion chemical ionization (PICI) was not observed. Alternatively, trifluoroethyl chloroformate was used for the derivatization of diamines with GC-NICI-MS detection [4], but no sensitivity enhancement was observed.

Other efforts to enhance ECNICI properties of AA include the use of pentafluorobenzyl (PFB) bromide to form PFB esters, with acetylation of the amine and silylation of side chain functional groups [5]. This method reported good sensitivity, but it involved three separate derivatization steps, and has not been applied to biological samples.

Derivatization of alcohols with chloroformates and GC-MS of the resultant carbonates also has been reported recently. Ethylene glycol in aqueous solution was derivatized with hexyl chloroformate [6, 7]. Elec-

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tron impact (EI) and PICI GC-MS were used for these analyses.

Pentafluorobenzyl chloroformate (PFB-chloroformate) has been used previously to enhance electron affinity in gas chromatography–electron capture detection (GC-ECD). Hartvig and co-workers [8–10] utilized the reagent for the determination of tertiary amines. The PFB carbamates were easily formed, highly stable and had excellent GC properties.

We report here the preparation and use of PFB-chloroformate as a derivatization reagent for enhancement of detection by ECNICI-MS. For this initial study, phenylalanine (Phe) and decanol were selected as model compounds. Aqueous solutions of Phe were derivatized under conditions similar to those of Husek [1] and Wang [2], whereas decanol requires heated anhydrous conditions for reaction. NICI mass spectra of the resultant derivatives are dominated by $[M - 181]^-$ ions that result from loss of the stable pentafluorobenzyl radical.

Experimental Section

Preparation of Pentafluorobenzyl Chloroformate

The method of Lee and Norton [11] was used without significant modification. (Safety note: All use of phosgene should be carried out in an appropriate chemical hood.) Pentafluorobenzyl alcohol (Aldrich Chemical, Milwaukee, WI; 10 g, 50.5 mmol) and *N,N*-dimethyl aniline (Aldrich; 8.35 mL, 65.6 mmol) in ethyl ether (40 mL) were added dropwise over 2 h to a 0 °C solution of phosgene (Fluka Chemical, Ronkonkoma, NY; 104 mL, 1.93-M toluene solution, 202 mmol) in ethyl ether (100 mL). After stirring overnight, the solution was concentrated under vacuum in a hood. The resulting yellow solution was diluted with ethyl ether (100 mL) and extracted with 3% HCl (100 mL), brine (100 mL), and dried over Na_2SO_4. The solution was concentrated and distilled to give 4.5 g of pentafluorobenzyl chloroformate (116–117 °C, 20 mm).

L-Phenylalanine was obtained from Sigma Chemical Co. (St. Louis, MO). Decanol, dimethylaminopyridine (DMAP) and pentafluorobenzoyl chloride (PFBzCl) were obtained from Aldrich. All solvents were of high performance liquid chromatography grade.

Derivatization

Phenylalanine was derivatized in a manner similar to that described previously [1, 2]. Briefly, a 10-μL aliquot of a phenylalanine solution in 0.1-M HCl was added to 100 μL of H_2O/ethanol/pyridine (60/30/10, v/v/v), and vortexed. PFB-chloroformate (10 μL, neat) was then added and the solution vortexed for 30 s. The reaction mixture was then extracted with 100 μL of toluene and an aliquot of the upper phase was transferred to an autosampler vial for GC-MS analysis. For characterization of the derivative, the concentration of Phe was 650 ng/μL. For selected ion monitoring (SIM) experiments, dilutions of the 650-ng/μL Phe stock were made to yield solutions at 650, 65 and 6.5 pg/μL in 0.1-M HCl. Aliquots of 0.1-M HCl were used as procedural blanks. Duplicates of diluted standards were then derivatized in the manner described above. One microliter injections were used for all analyses.

Decanol was derivatized by adding 25 μL of PFB-chloroformate to 50 μL of a 207-ng/μL CH_3CN solution; an additional 25 μL of CH_3CN was added to adjust the concentration for comparison with PFBzCl (following text). The reaction mixture was heated at 70 °C for 20 min and analyzed by GC-MS. A duplicate sample of decanol was derivatized with 25-μL PFBzCl (1% in CH_2Cl_2) and 25-μL DMAP (0.5% in CH_3CN).

The solution was heated at 70 °C for 20 min and analyzed by GC-MS.

Mass Spectrometry and Chromatography

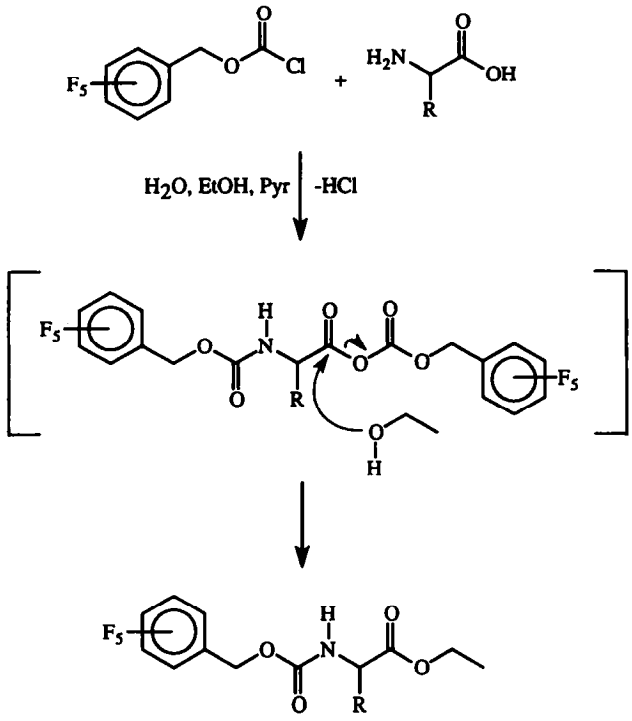
GC-MS analyses were performed on a Finnigan-MAT (San Jose, CA) TSQ-70 (scanning Q1) interfaced with a Varian Associates (Walnut Creek, CA) 3400 gas chromatograph, or on a Hewlett-Packard (Avondale, PA) 5989 interfaced with a HP-5890 Series II gas chromatograph. Ion source temperatures were 150 °C. Methane was used as the reagent gas for all chemical ionization experiments. SIM experiments of N-PFBC-Phe-OEt monitored m/z 236 $[M - 181]^-$ with a dwell time of 100 ms.

GC separations were performed on a HP-1 capillary column (Hewlett-Packard; 12-m × 0.2-mm i.d., 0.33-m film thickness) or a DB-5MS (J & W Scientific, Folsom, CA; 15-m × 0.25-mm i.d., 0.25-m film thickness). The oven was ramped from 70 °C to 300 °C at 30 °C/min, with a 1 min hold at 70 and 300 °C. Injection was splitless with a port temperature of 250 °C.

Results and Discussion

PFB-chloroformate reacts with phenylalanine in a manner similar to that reported for other chloroformate–AA derivatizations [1–3]. Under the conditions used in our experiments, the carboxylic acid portion of the molecule is converted to the ethyl ester, whereas the amino group is converted to the PFB carbamate. The reaction for a general AA is shown in Scheme 1.

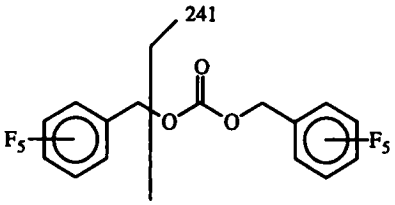
The advantage provided by the use of PFB-chloroformate is the addition of a highly electrophilic group to the analyte of interest, which then undergoes structure-specific cleavage under NICI conditions. This fragmentation yields a stable radical PFB · and a stable anion O(CO)NR. The structure of the phenylalanine derivative and the predominant fragmentation are shown in Figure 1.



N-PFBC-AA-OEt

Scheme I

The total ion chromatogram and NICI mass spectrum of the N-PFBC-Phe-OEt are shown in Figure 1. A large reagent-derived component in the total ion chromatogram is dipentafluorobenzyl (di-PFB) carbonate that results from hydrolysis of the chloroformate. The structure of the di-PFB carbonate is shown in Structure 1. Its NICI mass spectrum is dominated by m/z 241.



Dipentafluorobenzyl carbonate

Structure 1

Production of the di-PFB carbonate may involve intermediate reaction with pyridine that catalyzes its production. Evidence for this is found in the synthesis of PFB-chloroformate, which withstood acid washing with minimal formation of the di-PFB carbonate. *N,N*-Dimethylaniline, which was used as the base for the synthesis, was tested as the base for the PFB-chloroformate Phe reaction. However, the yield of the derivative formed with *N,N*-dimethylaniline was smaller than that produced by the use of pyridine as the base.

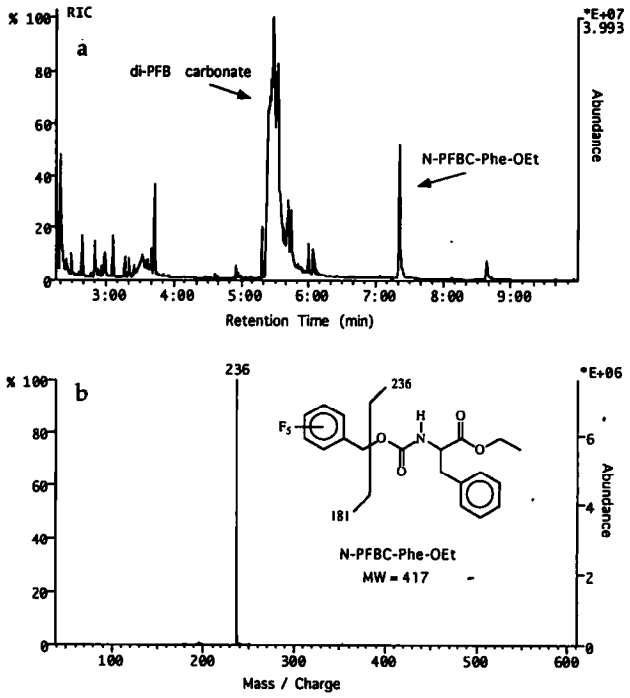


Figure 1. (a) Total ion chromatogram and (b) NICI mass spectrum of N-PFBC phenylalanine ethyl ester.

The di-PFB carbonate peak does not interfere with the analysis of the Phe derivative due to chromatographic as well as mass separation. The 65-pg Phe standard showed a signal-to-noise of 2:1 versus the blank. This standard corresponds to 400 fmol reacted and 650 fg injected (1 μL injected from a 100-μL toluene extract).

Previous work on ECNICI of AA–chloroformate derivatives attempted to introduce electron affinity via the alcohol that is incorporated as the ester [3]. Detection limits of 63 pmol were reported for glycine, alanine, valine, and leucine in the scanning mode. The NICI mass spectra, however, show extensive fragmentation, which dilutes the signal among several ions, precluding high sensitivity SIM.

We tested pentafluorobenzyl alcohol instead of ethanol in the reaction mixture with PFB-chloroformate and did form the N-PFBC phenylalanine pentafluorobenzyl ester (N-PFBC-Phe-OPFB). This supports the mechanism put forth by Wang, et al. [2] that the ester formation proceeds via formation of the mixed anhydride, which is then attacked by an alcohol. However, the molar response from N-PFBC-Phe-OPFB was smaller than that obtained from N-PFBC-Phe-OEt, in part due to extensive fragmentation of N-PFBC-Phe-OPFB (data not shown). We also compared methyl *t*-butyl ether, chloroform, and toluene as extraction solvents and found toluene gave the best response as measured by derivative peak area.

Our results from derivatization of decanol with PFB-chloroformate are promising, but suggest further optimization of reaction conditions is necessary. Electron impact analysis of the reaction mixture showed

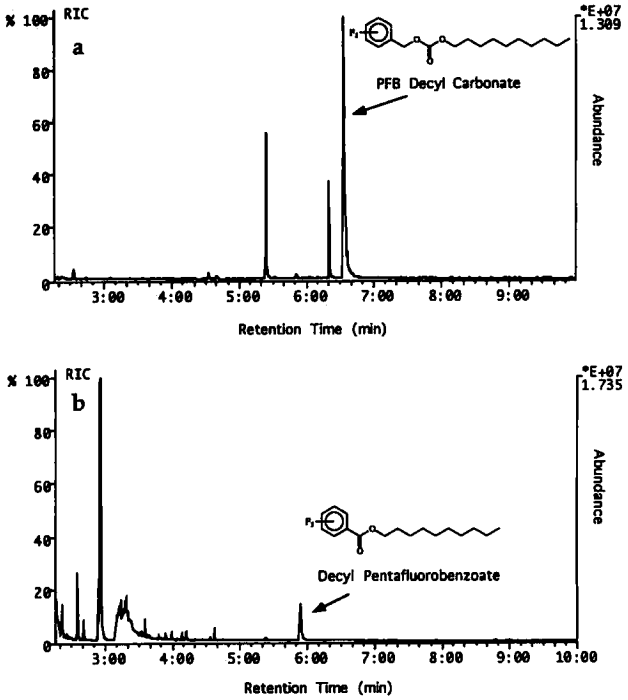


Figure 2. (a) NICI total ion chromatogram of the decanol–PFB-chloroformate reaction mixture. (b) NICI total ion chromatogram of the decanol–PFBzCl reaction mixture.

that substantial unreacted decanol remained which corresponded to a 20% yield. The PFB decyl carbonate exhibits remarkable sensitivity for ECNICI detection. The PFB decyl carbonate exhibits the same NICI fragmentation pattern exhibited by the N-PFBC-Phe-OEt, with essentially all the ion current residing in the $[M - 181]^-$ peak. Shown in Figure 2 is the TIC for the decanol PFB-chloroformate reaction mixture and in Figure 2, the TIC for the same amount of decanol derivatized with PFBzCl. Even with incomplete reaction the PFB decyl carbonate is a factor of 6 times more sensitive than the corresponding PFB ester. The NICI mass spectrum of PFB decyl carbonate is shown in Figure 3.

Conclusions

Pentafluorobenzyl chloroformate has been prepared and shown to derivatize amine and alcohol functionalities. Reaction of PFB-chloroformate with phenylalanine can be accomplished in aqueous solution in the presence of ethanol and pyridine. The N-PFBC-Phe-OEt derivative formed is thermally stable and exhibits excellent NICI properties. The N-PFBC-Phe-OEt $[M - 181]^-$ ion was detected easily at the femtomole level by SIM.

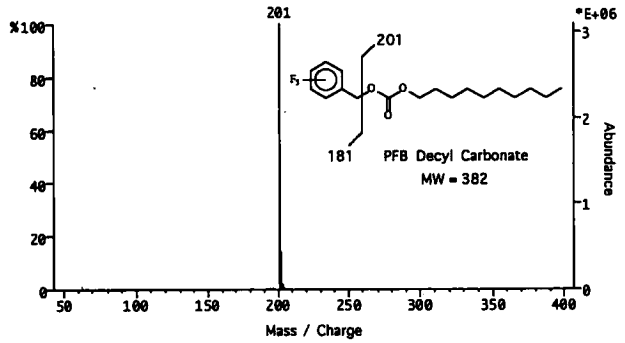


Figure 3. NICI mass spectrum of PFB decyl carbonate.

PFB-chloroformate is advantageous as a reagent for ECNICI-MS in that it incorporates electron affinity for enhanced electron capture. The derivatives fragment in such a way as to lose the stable PFB radical and form a structurally informative and stable carbamate or carbonate anion, as demonstrated by phenylalanine and decanol.

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